PATENT
Attorney Docket No.: 022101-001800US
Client Ref. No.: 19052-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Smith et al. Application No.: 09/823,649 Filed: March 30, 2001 For: HIGH TEMPERATURE REVERSE TRANSCRIPTION USING MUTANT DNA POLYMERASES Customer No.: 20350	Confirmation No. Examiner: Goldberg, J. Technology Center/Art Unit: 1634 DECLARATION UNDER 37 CFR § 1.132 OF DAVID H. GELFAND

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, David H. Gelfand, Ph.D., am Vice President of Discovery Research and Director of the Program in Core Research at Roche Molecular Systems. I am a co-inventor of the subject matter of the above-referenced patent application.

2. I hold a Ph.D. from the University of California San Diego, which was conferred in 1970. I have published more than forty-five scientific papers and book chapters and have received more than forty-five U.S. issued patents, particularly in the area of PCR and thermostable DNA polymerases. A copy of my curriculum vitae is attached as Exhibit 6.

3. I have read and am familiar with the contents of the above-referenced patent application and claimed subject matter. It is my understanding that the Examiner has rejected the claims for allegedly lacking enablement. This declaration is provided to show that DNA polymerases that, in their native form, comprise the motif defined by SEQ ID NO: 1 could be identified with routine methods at the time of the invention. In addition, using the teachings of the specification, one of skill could readily make and test mutants of these proteins for reverse transcriptase activity.

4. The structural and functional properties of thermoactive DNA polymerases were well-known in 2000, the effective filing date of the present application. Because of their use in PCR amplification assays and for DNA sequencing, thermoactive DNA polymerases had been the subject of study for over fifteen years at the time the present application was filed. At the time of filing, dozens of eubacterial thermoactive or thermostable DNA polymerase enzymes had been identified. Indeed, many high-resolution crystal structures of DNA polymerases derived from thermophilic microorganisms have been published. *See* Kim, Y., *et al.* 1995. *Nature* **376**:612; Eom, S.H., *et al.* 1996. *Nature* **382**:278; Kieffer, J.R., *et al.* 1997. *Structure* **5**:95; Keifer, J.R. *et al.* 1998 *Nature*. **391**:304; Li, Y. *et al.* 1998. *EMBO J.* **17**:7514; Li, Y. *et al.* 1998. *Prot. Sci.* **7**:1116; Hopfner, K-P., *et al.* 1999. *Proc. Natl. Acad. Sci., USA*. **96**:3600. Furthermore, a recent review of DNA Polymerase structure and fidelity (Beard, W.A. & Wilson, S.H. 2003. *Structure* **11**:489) highlights the structural conservation of motif elements in diverse DNA polymerases.

5. Based on these high resolution crystal structures, the structural features of DNA polymerases derived from thermophilic microorganisms were well known. Without wishing to be bound by theory, we believe that position 4 of the claimed motif is important to the reverse transcriptase activity of the enzyme because mutations at position 4 are likely to result in "tighter binding" of said polymerase to primer-template substrate.

6. Based on these known structural features, using standard sequence alignment programs described, for example, at page 13, lines 18-28 of the present application, one of skill could readily identify candidate thermoactive DNA polymerases. If necessary, one of ordinary skill in the art could easily verify whether a particular enzyme has thermoactive DNA

polymerization activity using simple experiments well-known to the art. For example, the ordinarily skilled artisan could identify that an enzyme has DNA polymerization activity by performing a primer extension assay. The thermostability of the enzyme can easily be tested by heating the enzyme before the assay. Alternatively, the ordinarily-skilled artisan could simply refer to the extensive literature to identify a suitable candidate enzyme for use in the methods of the invention. For example, *see* Lawyer, F.C., Stoffel, S., Saiki, R.K., Myambo, K., Drummond, R., and Gelfand, D.H. (1989). Isolation, Characterization, and Expression in *Escherichia coli* of the DNA Polymerase Gene from *Thermus aquaticus*. *J. Biol. Chem.*, **246**:6427-6437; Landre, P.A., Gelfand, D.H., and Watson, R.M. The Use of Cosolvents to Enhance Amplification by the Polymerase Chain Reaction. In: *PCR Strategies*. Eds. Innis, M.A., Gelfand, D.H., and Sninsky, J.J., (1995). Academic Press, San Diego, CA. pp 3-16; Abramson, R.D. Thermostable DNA Polymerases. In: *PCR Strategies*. *ibid*. pp 39-57; Abramson, R.A., Thermostable DNA Polymerases: An Update. In: *PCR Applications: Protocols for Functional Genomics*. Eds. Innis, M.A., Gelfand, D.H., and Sninsky, J.J., (1999). Academic Press, San Diego, CA. pp 33-48 and references therein.

7. The rejection appears to be based on a false assumption as to how one of skill would prepare enzymes useful in the claimed methods. To prepare such enzymes, one of skill would not synthesize and test 4 billion enzymes containing each of the species of motif as implied by the assertions at the top of page 6 of the Office Action. Rather, one of skill in the art would determine whether a previously identified thermoactive DNA polymerase comprises the motif defined by SEQ ID NO: 1 using the alignment algorithms noted above. Such sequence comparisons are entirely routine in the art. As demonstrated in the specification, we have identified thermoactive DNA polymerases from 12 different bacterial species that comprise the motif. See Table 1 at page, 12, lines 1-20.

8. After identifying a particular thermostable DNA polymerase that comprises the claimed motif, one of skill would then determine whether the thermostable DNA polymerase enzyme naturally comprises an appropriate residue at position 4 of the motif. If the thermostable DNA polymerase enzyme does not naturally comprise the appropriate residue at position 4 of the critical motif, the ordinarily-skilled artisan can routinely construct such a polymerase using, for

example, site directed mutagenesis protocols as described in the specification at page 14, lines 23-27. If the thermostable DNA polymerase enzyme naturally comprises an appropriate residue at position 4 of the critical motif, the ordinarily-skilled artisan will recognize that the thermostable DNA polymerase enzyme is suitable for use in the methods of the present invention without further alteration. Thus, one of ordinarily skill in the art can make thermostable DNA polymerases for use in the methods of the invention with no more than routine experimentation.

9. The Examiner is apparently concerned that because amino acid residues comprising the critical motif are not completely conserved among all DNA polymerases, the effect of changes to the amino acid residues other than position 4 would be unpredictable. One of skill would recognize that the lack of complete conservation within the claimed motif in thermoactive DNA polymerases is not critical to practicing the invention. As explained above, thermoactive DNA polymerases are well-characterized. The motifs and domains discussed above provide more than sufficient guidance as to which residues, if any, can be mutated for desired properties in the final enzyme. Moreover, the effect of any particular mutation can be readily tested in routine assays.

10. Moreover, we have prepared and tested two other species of Designer DNA polymerases in addition to the enzymes exemplified in the specification. As can be seen by the results described below, despite variability in positions inside and/or outside the motif identified here, we have shown that reverse transcriptase activity is enhanced by mutation at position 4 of the claimed motif.

11. Using standard techniques as described in the specification we have made and tested three additional DNA polymerases that have enhanced reverse transcriptase activity. Two of these novel Designer DNA Polymerases are ES112 and ES113. ES112 is the E683R mutant form of *Thermus* specie Z05 DNA Polymerase (SEQ ID NO: 11). ES113 is the E683K mutant form of *Thermus* specie Z05 DNA Polymerase (SEQ ID NO: 11). In both of these DNA polymerases the "X" residue at position 4 of SEQ ID NO: 1, or the "E" residue at position 4 in SEQ ID NO: 2 and SEQ ID NO: 3 have been mutated as taught in the specification. The third enzyme, CS6 DNA Polymerase, is a chimeric Designer DNA Polymerase comprising the DNA polymerase domain of *Thermotoga maritima* DNA Polymerase (SEQ ID NO: 15). CS6 DNA

Polymerase is more fully described in copending US Patent Application Serial No. 10/401,403, filed March 26, 2003 as SEQ ID NO:107 and in Fig. 5A. In CS6 DNA Polymerase, the "X" residue at position 4 of SEQ ID NO: 1 of the present specification is an arginine (R) and in SEQ ID NO: 5 the 4th residue is also arginine (R). All of these enzymes contain the "Critical Motif" as taught in Table 1 of the specification and all have improved reverse transcription (RT) capabilities.

12. Exhibit 1 ("Improved Mg^{2+}-activated RT-PCR with ES112 & ES113") shows a post-electrophoresis, ethidium bromide-stained and photographed agarose gel of various single enzyme RT-PCR products. The samples on the left were templated with a purified *in vitro* transcript RNA that corresponds to the *gag* region of HIV-1. The samples on the right were "template negative" reactions that did not have any HIV-1 template RNA. The data on the left show that the DNA polymerases ES112 and ES113 were able to generate robust yields of the expected amplicon (PCR product) when activated *either* with Mg^{2+} or Mn^{2+}. In striking contrast, *Thermus* specie Z05 DNA polymerase (the "wild-type control") is *only* able to generate the specific intended PCR product from an RNA template when activated with Mn^{2+} as the metal ion activator. Accordingly, both the ES112 and ES113 mutant DNA polymerases are magnesium-activated thermoactive and thermostable reverse transcriptases as well as possessing manganese-activated thermoactive and thermostable reverse transcriptase activity.

13. Exhibit 2 ("Reduced RT Time Requirement for ES112 & ES113 in Mn^{2+}") depicts "C_T" values *vs.* time of reverse transcription ("RT Incubation Time (min)") for three different DNA polymerase/reverse transcriptase enzymes. Reactions were set up and carried out similarly to what was described in Examples 2 and 3 of the specification. That is, real-time or "kinetic" fluorescence measurements were made at every extension cycle and the "C_T" values reflecting the accumulation of a specific amount of double strand DNA PCR product are plotted as a function of the reverse transcription time, which was varied. An increase in RT efficiency at shorter RT extension time results in reaching the threshold value in fewer cycles, *i.e.,* a lower "C_T" value. The data shows that when activated with magnesium ion ("Mg"), mutant Designer DNA polymerases ES112 and ES113 show a similar response to varying reverse transcription time as for *manganese* ion-activated ("Mn") *Thermus* specie (wild-type) Z05 DNA Polymerase.

This supports and extends the data in Exhibit 1 that both the ES112 and ES113 mutant DNA polymerases are magnesium-activated thermoactive and thermostable reverse transcriptases. Furthermore, as taught in the Specification on page 17, lines 19-21, the data in Exhibit 2 also show that when ES112 and ES113 mutant Designer DNA polymerases are activated with manganese ion, they both require much less RT time to achieve a similar low C_T value as *Thermus* specie (wild-type) Z05 DNA Polymerase, as little as 5 min in comparison to 30 min for *Thermus* specie (wild-type) Z05 DNA Polymerase. That is, both ES112 and ES113 mutant Designer DNA polymerases are improved and are much "faster" reverse transcriptases than *Thermus* specie (wild-type) Z05 DNA Polymerase. The improved reverse transcriptase enzymes of the invention could contribute significantly to a shorter time requirement for RT-PCR screening assays to detect important viral pathogens in blood (*e.g.*, not only HIV-1 but also Hepatitis C Virus [HCV], West Nile Virus [WNV], St. Louis Encephalitis Virus [SLV], coronavirus [SARS], *etc.*).

14. Exhibit 3 ("Efficient RT-PCR at Decreased ES112 & ES113 Enzyme Concentrations") shows the real-time or kinetic fluorescence measurements as a function of PCR cycle number for three different experiments. In Exhibit 3, the upper left panel depicts varying the concentration of *Thermus* specie (wild-type) Z05 DNA Polymerase. The lower left panel depicts varying the concentration of mutant Designer DNA polymerase ES112 and the upper right panel depicts varying the concentration of mutant Designer DNA polymerase ES113. The data clearly show a significantly delayed "C_T" for *Thermus* specie (wild-type) Z05 DNA Polymerase at 2.5 units per reaction (relative to higher concentrations of the enzyme) and in comparison with either ES112 or ES113 at 2.5 units/reaction, two additional Designer DNA Polymerase improved mutant reverse transcriptases of the invention. As taught in the Specification on page 17, lines 25-26, this series of experiments shows that a lower concentration of the improved reverse transcriptases of the invention is sufficient for efficient single enzyme RT and PCR.

15. Exhibit 4 ("Improved Low Copy Sensitivity with ES112 in Mn^{2+}-activated RT-PCR") presents the data from an experiment to support the teaching in the specification on page 16, lines 7-9 ("In Mn^{+2} reactions, the use of the mutant DNA polymerase provides for high

temperature reverse-transcription and amplification of RNA with a higher efficiency than achieved using the native enzyme."). In this experiment, manganese ("Mn^{2+}") activated reactions with wild type *Thermus* specie Z05 DNA polymerase (middle panels) were compared to magnesium ("Mg^{2+}") activated (top panels) or manganese ("Mn^{2+}") activated (bottom panels) reactions with mutant Designer DNA polymerase ES112. For each enzyme, 32-fold replicate *identical reactions* were set-up with on average about 0.5 – 0.7 copies of purified HIV-1 *in vitro* transcript RNA. This is a type of "mini Poisson analysis" in which differences in reverse transcriptase efficiency are readily detected. If cDNA is generated and amplified, RT-PCR assays are "positive." If cDNA is not efficiently generated, RT-PCR assays are "negative." This methodology sensitively distinguishes differences in reverse transcription efficiency. We analyzed the 96 PCR products (from each of the three 32-fold replicate RT-PCR assays) by "T_m" melting profile, using methods in the specification (page 25, lines 19-25). The T_m of the authentic HIV-1 RT-PCR product under these reaction conditions is about 80°C. In contrast, the T_m of non-specific or unintended side products (at these very-low-to-no target-present reactions) have a significantly lower and readily distinguishable T_m (about 73-77°C). The data in Exhibit 4 clearly show that the improved reverse transcriptases of the invention are characterized by improved reverse transcription efficiency. At these very low starting template concentrations (~ nominal 0.5 template copies/reaction), 10/32 of the wild type manganese-activated *Thermus* specie Z05 DNA polymerase were positive for HIV-1 amplicon (middle panels), identical to the number of positive reactions (10/32) when ES112 DNA polymerase was activated with magnesium (top panels). However, when Designer DNA Polymerase ES112 is activated with manganese (bottom panels), twice as many (20/32) of the HIV-1 RT-PCR assays were positive, indicating a significant increase in RT efficiency (compared to the wild type enzyme) when each is Mn^{2+}-activated. This increase in target detection sensitivity will likely be important in future diagnostic assays not only to monitor successful response to antiviral therapy (*e.g.*, for HIV-1, HCV, *etc.*) but as importantly for future blood screening assays in further reducing the possibility of an RNA virus-contaminated blood unit finding its way into the blood supply.

16. Exhibit 5 ("RT-PCR Using Mg^{2+}-activated CS6 DNA Polymerase") shows that CS6, a *Thermotoga maritima*-derived DNA polymerase domain chimeric enzyme with a

polymerase domain entirely unrelated to the *Thermus*-derived DNA polymerases described above, is also a magnesium-activated thermostable and thermoactive reverse transcriptase. Exhibit 5 shows a post-electrophoresis, ethidium bromide-stained and photographed agarose gel of several CS6-mediated, single enzyme RT-PCR products. CS6 DNA Polymerase is described in detail in copending US Patent Application Serial No. 10/401,403, filed March 26, 2003 as SEQ ID NO:107 and in Fig. 5A. The "Critical Motif" (Table 1), SEQ ID NO: 1 and SEQ ID NO: 5 of the present specification are found uniquely at CS6 DNA Polymerase amino acids 741 through 751. Note that position 4 of the "Critical Motif" (Table 1) in CS6 DNA Polymerase is not glutamic aid (E) and is arginine (R). For the experiment in Exhibit 5, purified, *in vitro*-transcribed AW109 cRNA was used at different input copy numbers (10^6, 10^5, 10^4, 10^3, or none) in duplicate reactions with CS6 DNA Polymerase and magnesium ("Mg^{2+}") activation. The results clearly demonstrate that thermophilic and thermostable Designer DNA Polymerase CS6 is an improved, magnesium-activated reverse transcriptase. Furthermore, the "Critical Motif" (Table 1 in the specification) shows that the majority of the amino acids (6 of the 11 positions) are different between many of the *Thermus* genus enzymes and the *Thermotoga* genus enzymes. Nevertheless, when the teachings of the specification are followed, improved reverse transcriptases are obtained. Finally, there is a great deal of amino acid sequence divergence between the *Thermus* genus DNA polymerases and the *Thermotoga* genus DNA polymerases, reflecting the considerable evolutionary divergence of the microorganisms from which the

enzymes originally derive. Indeed, there is only about 45% overall amino acid identity between aligned DNA polymerase sequences from representatives of the *Thermus* genus and representatives of the *Thermotoga* genus. That is, more than half of the amino acids are different. However, SEQ ID NO:1 and the "Critical Motif" (Table 1) of the invention can readily be uniquely identified in the DNA polymerase domains of these microorganisms' DNA polymerases.

17. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon.

Date: April 21, 2004

By: David H. Gelfand, PhD.

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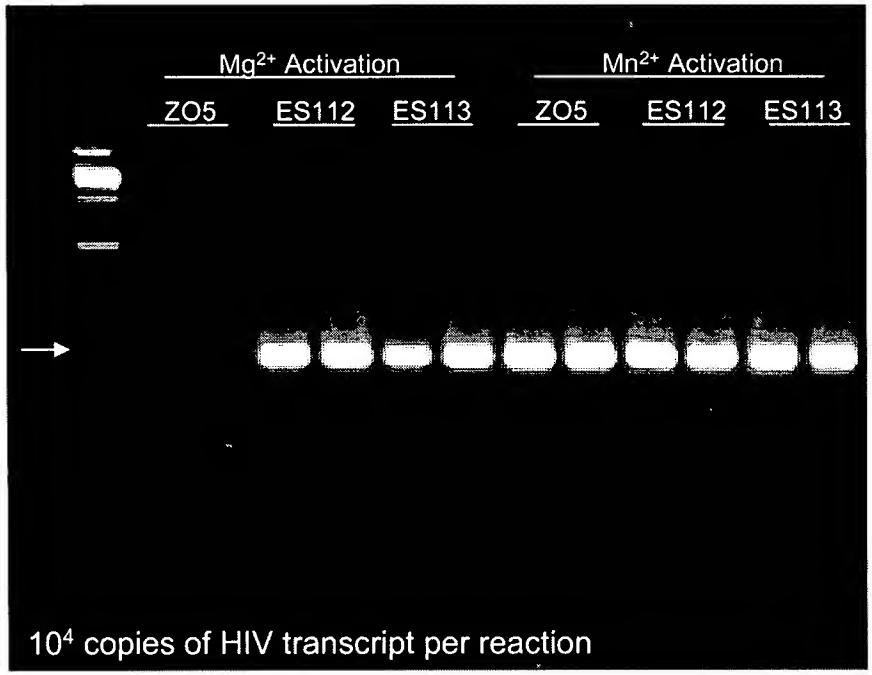
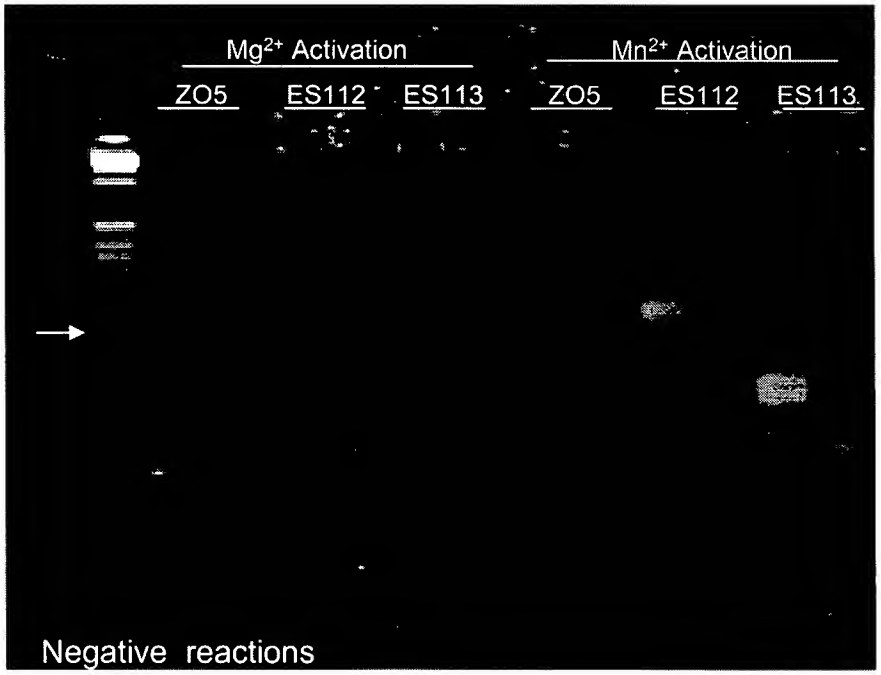
Improved Mg^{2+}-activated RT-PCR with ES112 & ES113

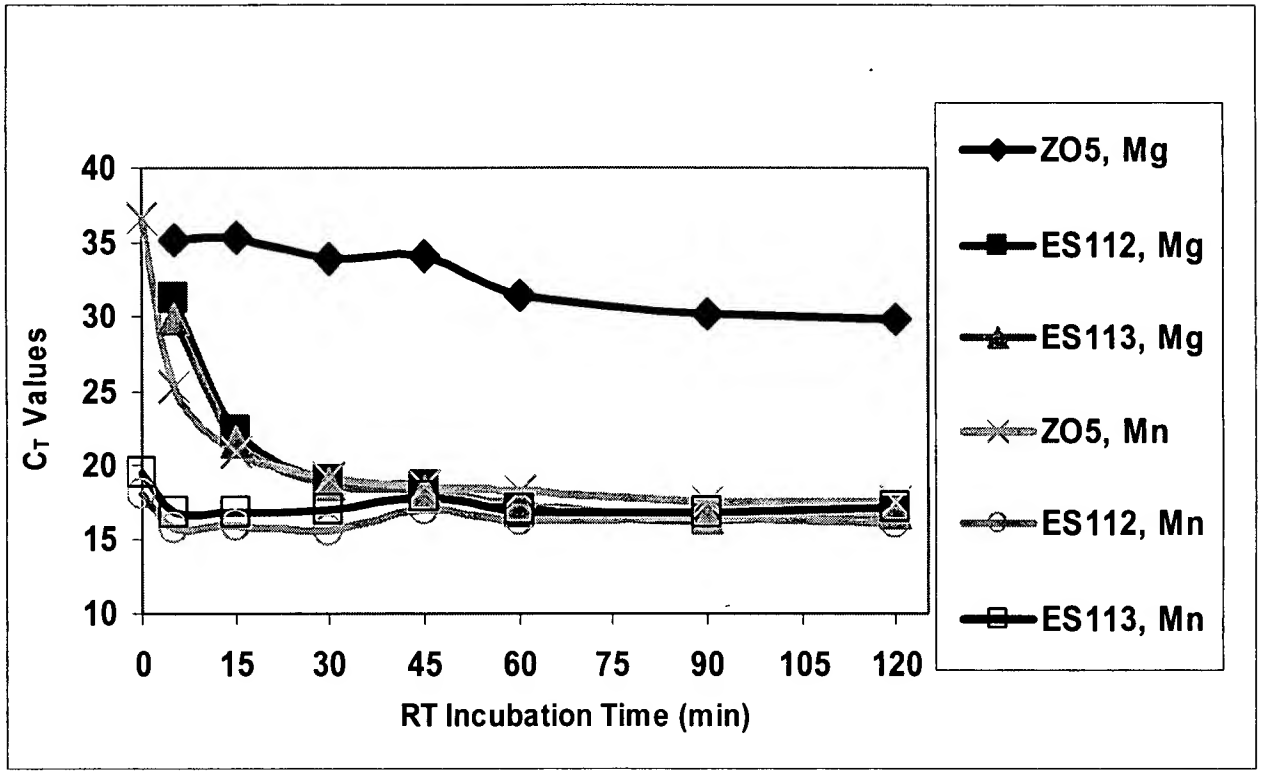




Three different thermostable DNA polymerases were used to reverse transcribe an HIV transcript RNA template and subsequently amplify the cDNA in a coupled RT-PCR in the presence of either 3 mM Mg^{2+} or 3 mM Mn^{2+}. After 55 cycles of PCR, gel results demonstrate specific amplification products from RNA with ZO5 in the presence of Mn^{2+}, but no specific product was observed when Mg^{2+} was used as the divalent metal ion activator. However, designer enzymes ES112 and ES113 produced specific amplification product with either Mg^{2+} or Mn^{2+} activation.

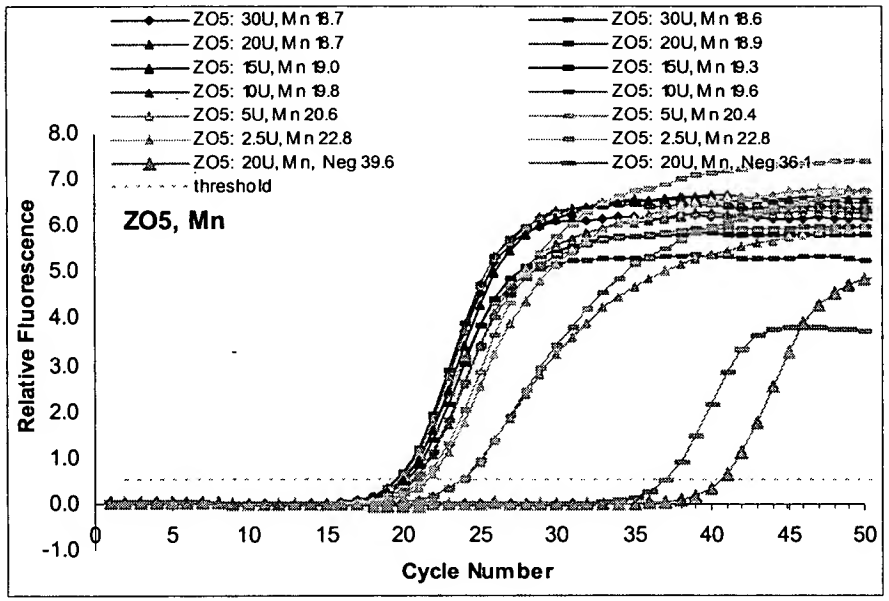
Reduced RT Time Requirement for ES112 & ES113 in Mn^{2+}

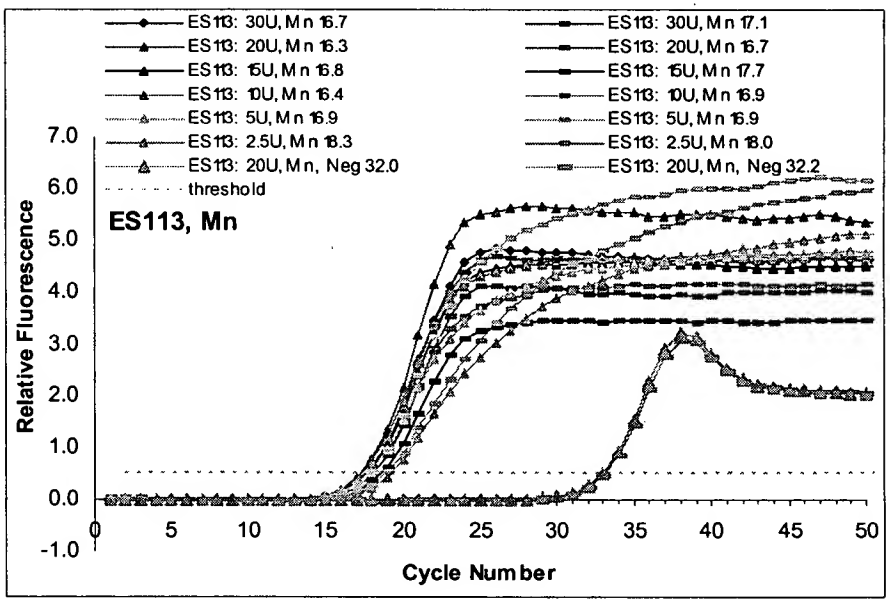
A 280 bp GAPDH RNA template was subjected to various RT incubation times and then amplified by PCR. In all cases PCR profiles were identical and the results were analyzed by kinetic PCR. The C_T values of growth curves are plotted in the following chart:

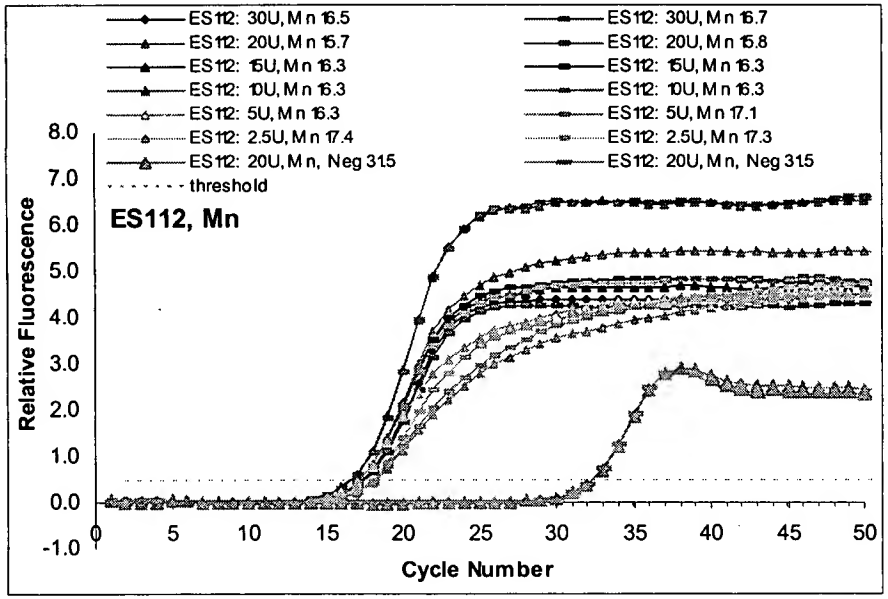


Following a 30 min RT incubation time and Mg^{2+} activation, the mutant enzymes ES112 and ES113 achieved RT activity similar to Mn^{2+}-activated wild-type ZO5 DNA polymerase. With Mn^{2+} activation, the mutant enzymes exhibited similar RT activity, but with much shorter RT incubation times (as low as 5 min). Even with no added RT incubation time there were only slight C_T delays for Mn^{2+}-activated mutant enzyme amplifications and initial PCR ramp times apparently are sufficient for the RT step to occur.

Efficient RT-PCR at Decreased ES112 & ES113 Enzyme Concentrations

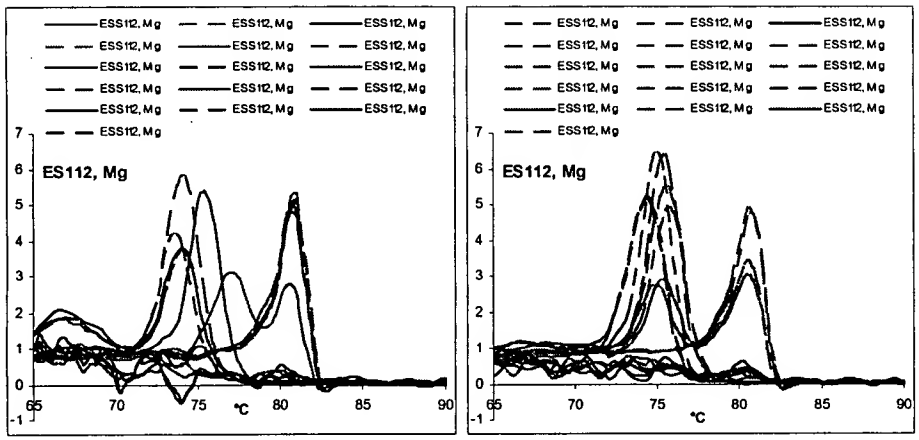






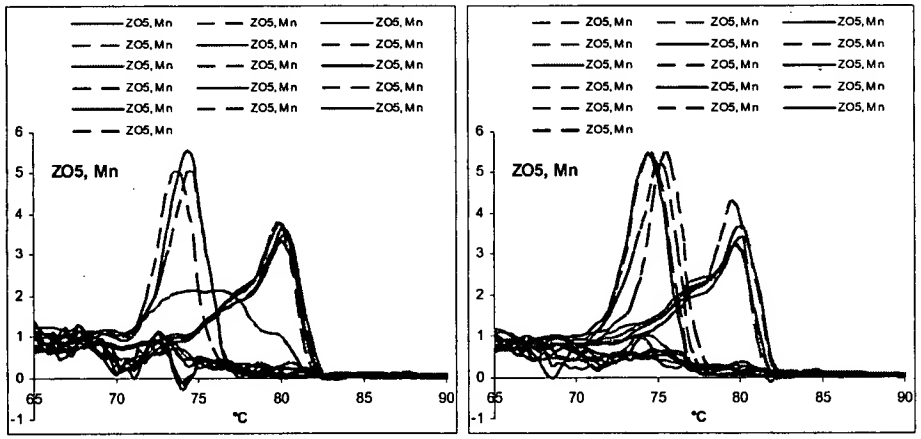
Enzyme concentration was titrated from 30 U down to 2.5 U per reaction for ZO5, ES112 and ES113. A significantly higher C_T value is observed with 2.5 U of ZO5 when compared to higher enzyme concentrations. The ES112 and ES113 perform relatively efficient RT-PCR with as little as 2.5 U of enzyme per 50 µL reaction.

Improved Low Copy Sensitivity with ES112 in Mn^{2+}-activated RT-PCR



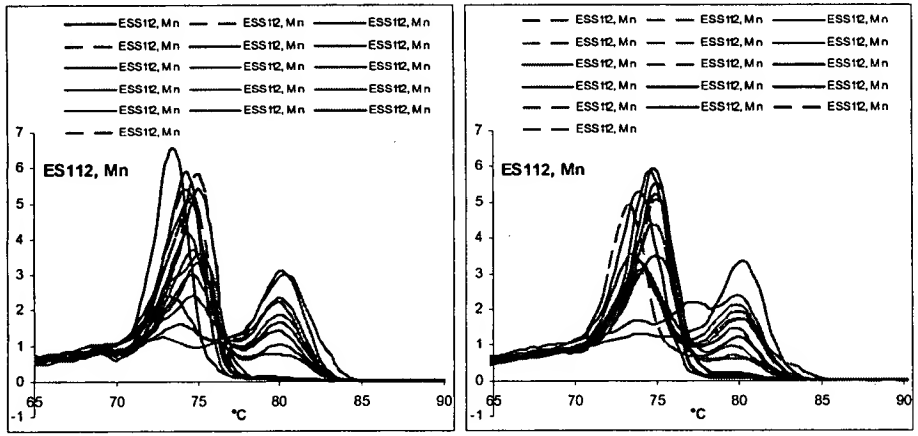
ES112, Mg^{2+}

10/32 Positives



ZO5, Mn^{2+}

10/32 Positives

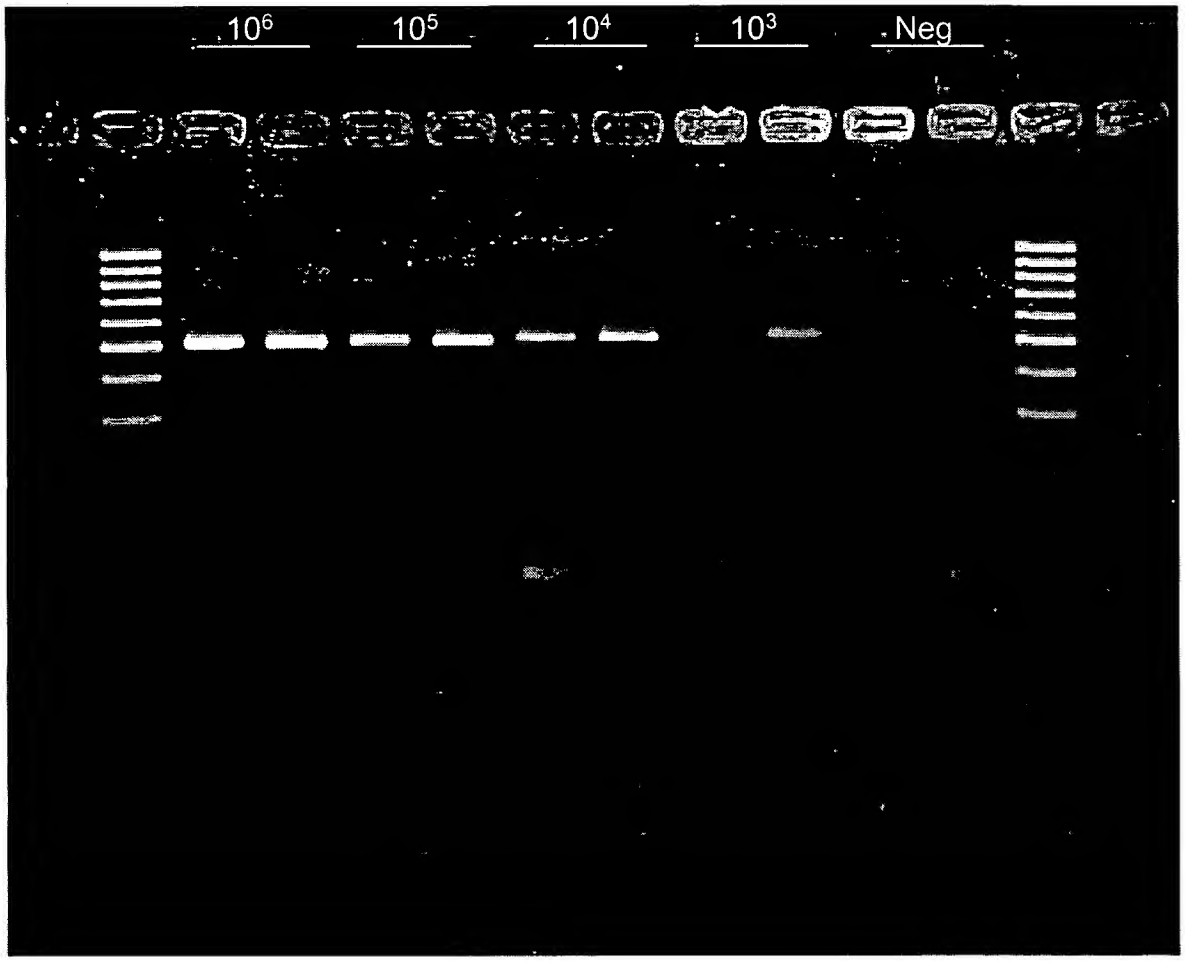


ES112, Mn^{2+}

20/32 Positives

Nominally 0.5 copies of HIV transcript RNA per reaction were amplified in 50 µL RT-PCR amplifications optimized for Mg^{2+}-activated ES112, Mn^{2+}-activated ES112 or Mn^{2+}-activated ZO5 ("Gold Standard"). The T_m of end-point RT-PCR product was used to distinguish successful amplification of transcript RNA (specific product) from negative reactions (nonspecific product). The Mg^{2+}-activated ES112 reactions had the same low copy sensitivity as the Mn^{2+}-activated ZO5, while the low copy sensitivity was observed to be twice as good with Mn^{2+}-activated ES112.

RT-PCR Using Mg^{2+}-activated CS6 DNA Polymerase



Various concentrations of pAW109 transcript RNA were amplified by single-buffer RT-PCR. All reactions contained 2 mM Mg^{2+} and CS6 DNA polymerase. Following 45 cycles of PCR, products of the correct size were observed with as little as 10^3 copies of RNA per reaction. Negative control reactions lacking RNA transcript produced no specific product of the expected amplicon size.

CURRICULUM VITAE

David H. Gelfand

Personal Statistics

Date of Birth:	June 9, 1944
Place of Birth:	New York, New York

Education

1970 Ph.D.	Biology, University of California, San Diego, La Jolla, California
1966 A.B.	Biology, Brandeis University, Waltham, Massachusetts

Research and Professional Experience

12/91 - Present 12/00 – Present	Director, Program in Core Research Vice President, Discovery Research Roche Molecular Systems, Inc. 1145 Atlantic Avenue Alameda, CA 94501-1145
11/88 - 12/91	Director, Core Technology, PCR Division, Cetus Corporation
3/81 - 12/91	Vice President, Scientific Affairs, Cetus Corporation
1/79 - 3/81	Vice President and Director of Recombinant Molecular Research, Senior Scientist, Cetus Corporation
12/76 - 10/79	Director, Recombinant Molecular Research Cetus Corporation
8/76 - 1/77	Assistant Research Biochemist, University of California at San Francisco San Francisco, CA
Sponsor:	William J. Rutter, Professor
Project:	Isolation, characterization and expression of eucaryotic DNA sequences in bacterial cells.
1/72 - 8/76	Assistant Research Biochemist and Laboratory Manager, University of California at San Francisco, San Francisco, California
Sponsor:	Gordon M. Tomkins, Professor (deceased July 1975)
Project:	Effect of oncogenic viral transformation on the regulation of gene expression in cultured mammalian cells.
	Isolation and characterization of mutants defective in tyrosine aminotransferase activity.
	Construction of hybrid DNA molecules and genetic transformation.
7/70 - 1/72	Research Associate in Biology, University of California at San Diego, La Jolla, CA
Sponsor:	Masaki Hayashi, Associate Professor
Project:	DNA-dependent RNA-directed protein synthesis *in vitro*: temporal control of transcription and translation.

5/70 - 7/70	NIH postdoctoral trainee in Molecular Genetics, University of California at San Diego, La Jolla, California
Sponsor:	Masaki Hayashi, Associate Professor
Project:	Same as above.
10/66 - 5/70	NIH predoctoral trainee in Molecular Genetics, University of California at San Diego, La Jolla, California
Sponsor:	Masaki Hayashi, Associate Professor
Project:	Viral DNA-dependent protein synthesis
7/66 - 10/66	Research Associate in Biology, University of California at San Diego, La Jolla, CA
Sponsor:	Stanley Mills, Professor
Project:	Passive immune kill in HeLA cells *in vitro*.
6/65 - 9/65	Research Assistant in Biochemistry, Brandeis University, Waltham, Massachusetts
Sponsor:	Gordon Sato, Associate Professor
Project:	Mechanism of steroid production and secretion in mouse tumor cells *in vitro*.
6/62 - 9/62	Research Assistant, School of Medicine, University of Michigan, Ann Arbor, Michigan
Sponsor:	Raymond H. Kahn, Professor
Project:	Effect of *Tubercule bacilli* in chick embryonic lung tissue *in vitro*.
6/61 - 9/61	Research Assistant, Department of Biology, New York University, New York, New York
Sponsor:	M. J. Kopac, Professor
Project:	Establishment of primary cell lines of amphibian liver *in vitro*.

Awards and Honors

New York State S.E. Regional Science Fair, First Prize winner, Senior Division Biology and Grand Prize Winner (1962).

New York State Science Fair Finalist Sixth Prize (1962).

Awarded New York State four-year full-tuition scholarship (award not accepted).

Recipient, May 1990, IPO "Distinguished Inventor Award," Senate Office Building.

Memberships

American Association for the Advancement of Science

American Society of Biochemistry and Molecular Biology

American Society of Microbiology

Genetics Society of America

National Science Foundation Scientific Advisory Council (1981-1984)

Department Visiting Committee, Department of Microbiology, University of Texas, Austin (1988-)

Publications

1 **Gelfand, D.H.,** and Hayashi, M. (1969). Electrophoretic characterization of ΦX174-specific proteins. *J. Mol. Biol.*, **44**:501-516.

2 **Gelfand, D.H.,** and Hayashi, M. (1969). DNA-dependent RNA-directed protein synthesis *in vitro*, II: Synthesis of a ΦX174 coat protein component. *Proc. Natl. Acad. Sci. USA*, **63**:135-137.

3 Bryan, R.N., **Gelfand, D.H.,** and Hayashi, M. (1969). Initiation of SV40 DNA-directed protein synthesis with N-formylmethionine *in vitro*. *Nature*, **224**:1019-1021.

4 **Gelfand, D.H.,** and Hayashi, M. (1970). DNA-dependent RNA-directed protein synthesis *in vitro*, IV: Peptide analysis of an *in vitro* and *in vivo* ΦX174 structural protein. *Proc. Natl. Acad. Sci. USA*, **67**:13-17.

5 Jeng, Y., **Gelfand, D.H.,** Hayashi, M., Schleser, R., and Tessman, E.S. (1970). The eight genes of bacteriophages ΦX174 and S13 and comparison of the phage-specific proteins. *J. Mol. Biol.*, **49**:521-526.

6 **Gelfand, D.H.** (1970). Viral DNA-Dependent Protein Synthesis. Ph.D. dissertation.

7 **Gelfand, D.H.,** and Hayashi, M. (1970). *In vitro* synthesis of a DNA-dependent RNA polymerase coded on Coliphage T7 genome. *Nature*, **228**:1162-1165.

8 Rousseau, G.G., Higgins, S.J., Baxter, J.D., **Gelfand, D.H.,** and Tomkins, G.M. (1975). Binding of glucocorticoid receptors to DNA. *J. Biol. Chem.*, **250**:6015-6021.

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Issued U.S. Patents

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2. **Gelfand, D.H.**, Chang, S., and Wong, H.C. "Polypeptide Expression Using a Portable Temperature Sensitive Control Cassette with a Positive Retroregulatory Element." U.S. Patent No. 4,666,848 assigned to Cetus Corp. 5/19/87.

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